# Biol 1C - Modules 7.1 & 7.2

## Before Class

### Activity 1:  Watch two expert group presentations

Take notes in the table below and be sure to include a question you have for each group about their intervention strategy.

| | **Video 1** | **Video 2** |
|---|---|---|
| Presentation title | Creating GMO mosquitoes to persist in wild populations | Developing antimalarial drugs |
| Summary of intervention | Modifying mosquitoes by creating GM anopheles stephensi lines. Researchers used the carboxypeptidase (Cp) or vitellogenin (Vg) promoters to create mosquito lines with upregulated mosquito immunity genes in the midgut after a blood meal. | Inhibitor will pass by the red cell membrane, PV membrane, merozoite membrane, and exonome membrane to inhibit sub1 from its initial spot inside. Inhibitor attaches to a sub1 protein. Maintenance of the asexual blood stage, treated with peptic acid inhibitor to see what effects this acid on the asexual blood stage of the parasite. In order to determine which peptic acid inhibitor was the best researchers used covalent molecular docking to test inhibitor force |
| Stage in the *Plasmodium* life cycle that was interrupted | Once monocytes are ingested from an infected human, the monocytes enter the midgut where it will create a zygote and cause a reproduction of plasmodium within the mosquito. This stage occurs in the gut. Genetically modify genes in the mosquito midgut, specially the anopheles mosquito defensive line (primary mosquito vector of malaria) to strengthen the immune system against plasmodium | Step 4 of the plasmodium life cycle: merozoites will enter the red blood cells and reproduce asexual in the PP. Parasite cyclic GMP kinase is activated and sub1 protein is discharged from the exonemes. The sub1 will activate the PV surface protein and it will rupture. Cell will open and new generation is released |
| Question for the group | How does the conditions in which the female mosquitoes were put in (septic vs. aseptic) a way to | How is the kinetics of binding related to the cleavage of SERA5 that causes inhibition of PfSUB1 |

| | | |
|---|---|---|
| | demonstrate the female mosquito's mating preference? | (enzyme involved in the emergence of malaria in the parasite red blood cells)? |

## Activity 2: Notes on Madagascar Malaria paper

List all (at least 5) possible factors contributing to the Madagascar malaria epidemic discussed in the Kesteman *et al.* paper. As you are reading the paper, use the table below to keep track of the factors as you encounter them. Make sure to read through the discussion carefully, as many factors that might have contributed to the disease outbreak are highlighted there. *Hint: take a look at Table 1 in the paper.*

| **Factor** | **Organism(s) affected** |
|---|---|
| Increase in rainfall | Mosquitoes - more space for eggs/larvae |
| Intensive control interventions deployed in the previous decade | Population's immunity against clinical malaria |
| Less intensive control interventions | Parasite- made possible for the parasite to keep circulating and the number of cases to grow |
| Poor bio-efficacy of a subsample fo LLINs | Protective effect of the LLINs at community level- impaired this protective effect by the reduction of endophagic and endophilic vectors -> more malaria cases |
| Experience of stock-outs in ACT and/or RDTs in health facilities | Infected individuals- treatments were not optimal during stock outs |
| Political and economic crisis | Population of Madagascar- reduction in healthcare workforce, especially marked in rural areas |
| Waning bed net use | People in the population- not having a nightly bed net used was significantly associated with higher parasite prevalence |

Copy and paste BOTH completed tables, from Activity 1 AND Activity 2, into the pre-class homework assignment for Module 7.1.

# During Class

## Announcements

## Group Activity Overview:  Jigsaw groups malaria treatment plan

The goal of **this 2-day activity** is for you to synthesize the molecular/cellular/ecosystem level details of the expert strategies you researched last week and create a presentation in which you share what you learned with a diverse group of experts, to create a plan to combat a future malaria disease outbreak. You will use the systems-level tools you developed over this quarter of BIOL 1C to predict the effects of *at least two* of these intervention strategies on the malaria disease triangle and malaria SIR model.

Your Mission: You have been put on a team of diverse malaria expert scientists at the President's Malaria Initiative.  Each member of your team is a scientist with expertise in a different malaria treatment intervention strategy.  Together, your team will build and motivate a multi-pronged treatment plan to limit the spread of malaria in Madagascar.  A team of scientists recently conducted some on-the-ground experiments and interviews in Madagascar, and their results are reported in the Kesteman *et. al.* paper.

On Wednesday, you will prepare and submit a treatment plan strategy and its predicted effects on population dynamics grounded in SIR modeling. Use a Google slideshow presentation (without audio; just 6 slides using the prompts as shown in the Lecture Slides "Components of Your Malarial Treatment Plan") to communicate the following elements of your plan:

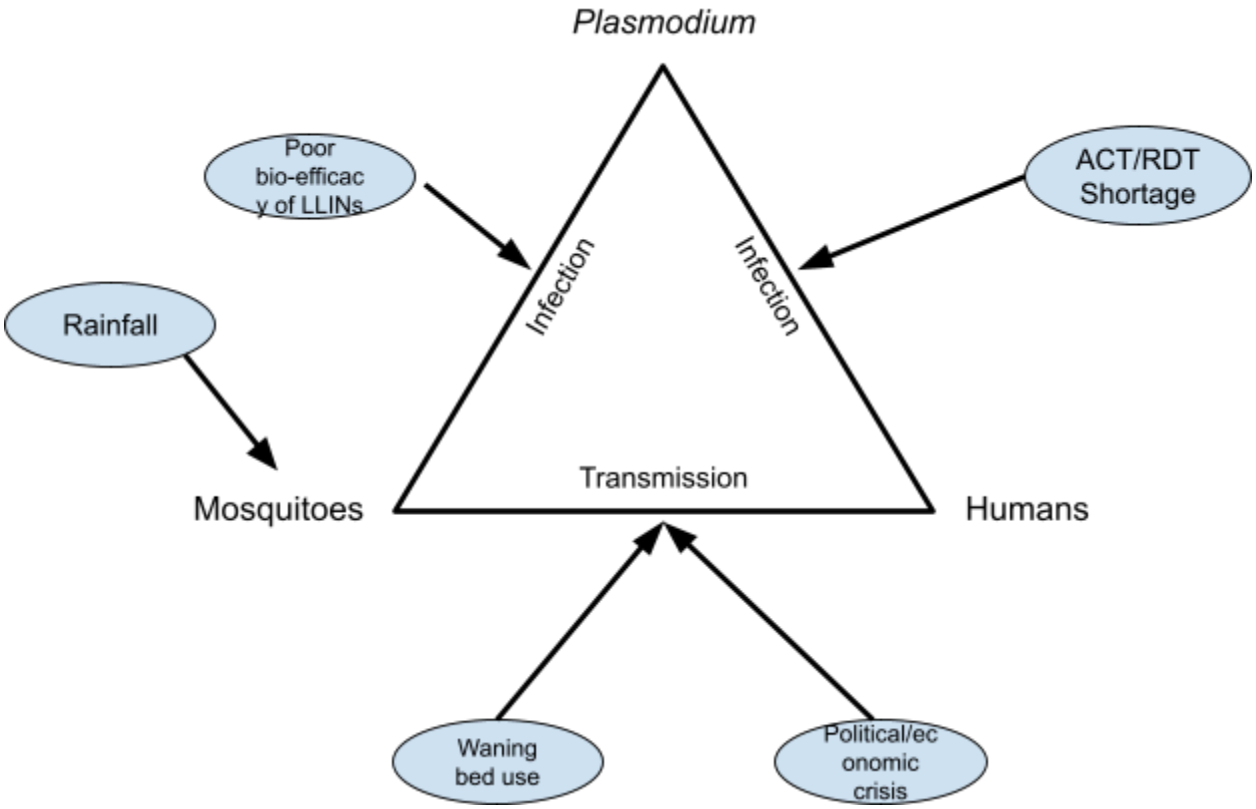
1) Title Page with descriptive title and name of researchers on your diverse expert team and their respective expertise
2) Methods of Intervention: Include rationales for halting/slowing the malaria epidemic and pros/cons for the two interventions your team will address (Activity 5).
3) Treatment strategy illustrated in a malaria triangle diagram (Activity 5):  Include effects that caused the malaria outbreak in Madagascar and at least two treatment interventions that would reverse future outbreaks. Justify connections between your interventions and the triangle.
4) SIR diagram with interventions highlighted (Activity 6):  Include new variables as needed and make sure to define any new variables you use.
5) Modeling results from your team's Python simulations (Activity 7) with and without the intervention(s).
6) References:  Include all relevant sources used in APA format

Use the prompts below to organize your group's research about your case study and treatment plan. If you haven't already, copy and share a version of this worksheet (or start with the original version) with your group members.

When it is time to submit your results: In Camino, find People > Groups > "Jigsaw Groups" and you will move your team into the appropriate group number indicated for 9:15 or 10:30 lecture class. Convert your google slides to PDF and upload for your team when you have completed this activity. This activity is part of the malaria project grade.

## Activity 3: Madagascar Malaria Disease Triangle

Build a malaria disease triangle (human-mosquito-parasite) that includes the causative factors you listed above. Use arrows or T-stops (arrow with a box) to indicate whether each factor promotes or inhibits the three species or the interactions between them. Double-click to edit.




## Activity 4: Expert group summaries

As a group, take turns sharing the information you learned in your expert group presentations. Consider the different treatments discussed in your expert groups in Week 9. As one person

shares, take turns recording what they said. (Do not just divide and conquer here - the point is to share what you learned with the group). Describe the pros and cons of the treatment/intervention and how they relate to the factors above. If you have two experts with the same expertise, you may decide to work together or independently (add another row to the table if needed) for this section of the activity. If there is not a person in your group that is an expert on a given topic, you may skip that intervention type.

<table>
<tr><td colspan="2">Vaccines: Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso<br><br>Recorder’s name: Bella Matusich<br>Describe the intervention strategy here:<br><br>They created a protein that would code for the gene R21. This gene then enters the nucleus of a yeast cell, then the yeast cell would express the function of R21. R21 would intercept the cycle before the red blood cells are infected.</td></tr>
<tr><td>Pros<ul><li>R21 appears to be safe and immunogenic for African children</li><li>Shows high level efficacy</li></ul></td><td>Cons<ul><li>Vaccine could cause fever, nausea</li><li>It requires multiple doses–making it harder for more people to access it</li></ul></td></tr>
<tr><td colspan="2">Antimalarial drugs: [technique, biology]<br>Recorder’s name:<br>Describe the intervention strategy here:</td></tr>
<tr><td>Pros<ul><li></li></ul></td><td>Cons<ul><li></li></ul></td></tr>
<tr><td colspan="2"><u>GMO mosquitoes:</u> Genetically modifying genes in the midgut to increase the immunity of mosquitos against the Plasmodium bacteria.<br><u>Recorder’s name:</u> Fatima Laureano-Maravilla<br><u>Describe the intervention strategy here:</u> There was the use of CpRel215 and CpDsPfs3 considered to have the most resistance against the Plasmodium bacteria and were tested for the prevalence of modification by studying their mating preferences.</td></tr>
<tr><td>Pros<ul><li>High persistence and spread of genetically modified genes in the population</li><li>Resistance is very stable over time</li><li>Genetically modified mosquitos have a higher fitness</li><li>35% transmission-blocking effect would eliminate hypoendemic malaria</li></ul></td><td>Cons<ul><li>Wasn’t field tested, results could be different in nature</li><li>GM mosquitos only have incomplete resistance to Plasmodium</li><li>Couldn’t be a malarial control strategy on its own</li><li>Negative public opinion on GMOs</li></ul></td></tr>
</table>

**Human ↔ Mosquito Interactions:** [technique, biology]
Recorder's name: Ethan Baker
Describe the intervention strategy here:
Use of bed nets without insecticides and instead using cones that trapped mosquitoes where they would die from dehydration.

| **Pros** | **Cons** |
| --- | --- |
| ● Had a higher mosquito mortality rate<br>● Doesn't include insecticides that mosquitoes can evolve to be immune to so there is no need to continue to change the insecticides used<br>● Similar cost | ● Regulatory constrictions<br>● Waste<br>● Maintenance |

**Control mosquito populations:** [technique, biology]
Recorder's name: Isabella Enriquez
Describe the intervention strategy here:
Ultimately, this paper is about focusing on genetically modified mosquitoes of this particular species. The intervention being proposed targets the vector of the disease triangle since researchers are going to test the suppression of female fertility in Aedes aegypti mosquitoes through CRISPR. CRISPR is a mechanism that targets the B2t gene present in the testes of the male Aedes aegypti mosquitoes in order to sterilize them and cause infertility for the females when they mate so that these female mosquitoes are no longer able to transmit disease. The strategy being proposed which targets the vector of the disease triangle is used to control mosquitoes that cause dengue, furthermore this strategy can be used for the anopheles mosquitoes which cause malaria. In this study, scientists used CRISPR to knock out the B2t gene since it is required for male fertility in *Ae. aegypti.* GFP used to confirm knockout through CRISPR. Disruption of the B2t gene proven by GFP expression. GFP is under the same promoter as B2t so expression would indicate disruption of B2t coding sequence. Proven perturbation of B2t gene, creating the B2t1 mutant allele.

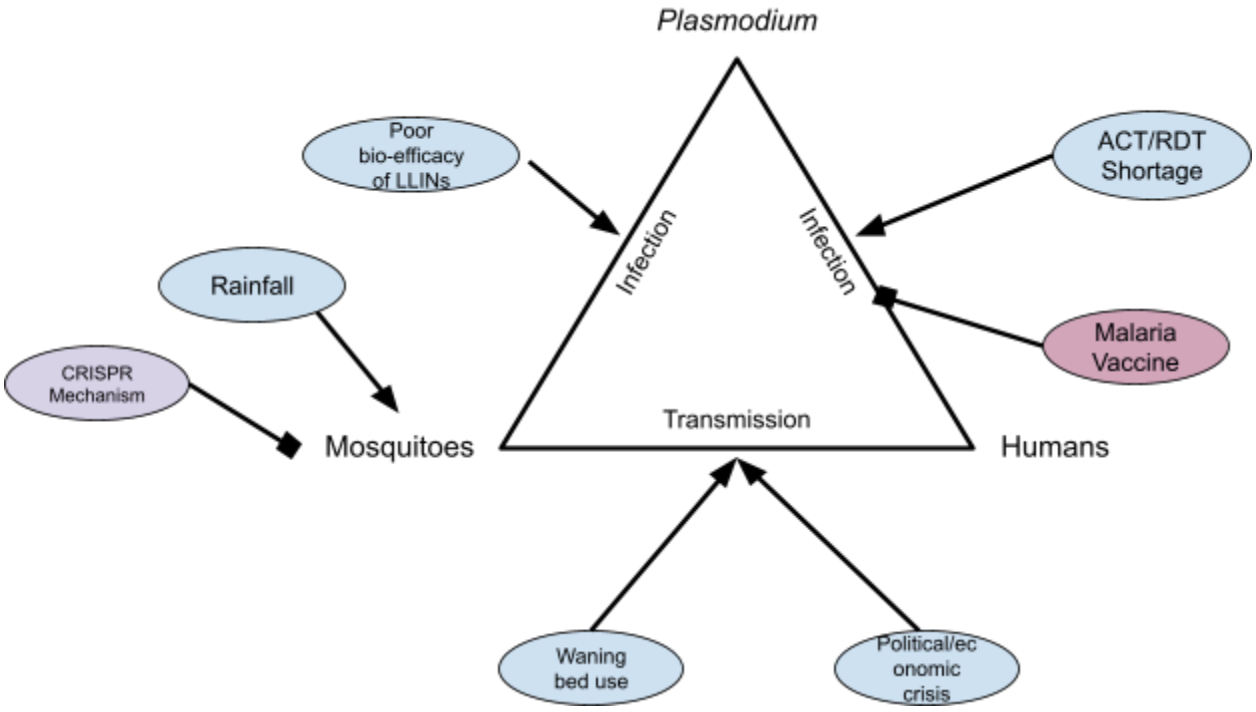
| **Pros** | **Cons** |
| --- | --- |
| ● Successful in identifying B2t gene as effective target<br>● Doesn't use harsh chemicals or radiation that could linger in the environment<br>● Potentially very effective in controlling mosquito population<br>  ○ Strong results<br>● This could be used in other types of mosquitoes | ● Expensive<br>● Difficult to genetically modify entire population<br>● Implications of ending an entire species population<br>● Can't propagate sterile mosquitoes<br>  ○ Gene does not end up in the population |

Based on the indicated factors that lead to the outbreak you identified above, discuss and then select at least two specific treatment options that would be appropriate to combat a future disease outbreak. As a group, decide which of the treatment strategies above will be most effective in your solution to the outbreak problem. List your two chosen options below that your group will move forward with:

1. Malarial Vaccine: Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso
2. Control Mosquito population: Scientists used CRISPR to knock out the B2t gene since it is required for male fertility in *Ae. aegypti.*

## Activity 5: Madagascar treatment strategy disease triangle

Add each treatment strategy that your group will focus on to the malaria triangle diagram that you began in Activity 2. Copy and paste your Google Drawing from Activity 2 below. Add your treatment strategies in a different color, and again use arrows or T-stops to indicate whether each factor promotes or inhibits the species or the interactions between them.



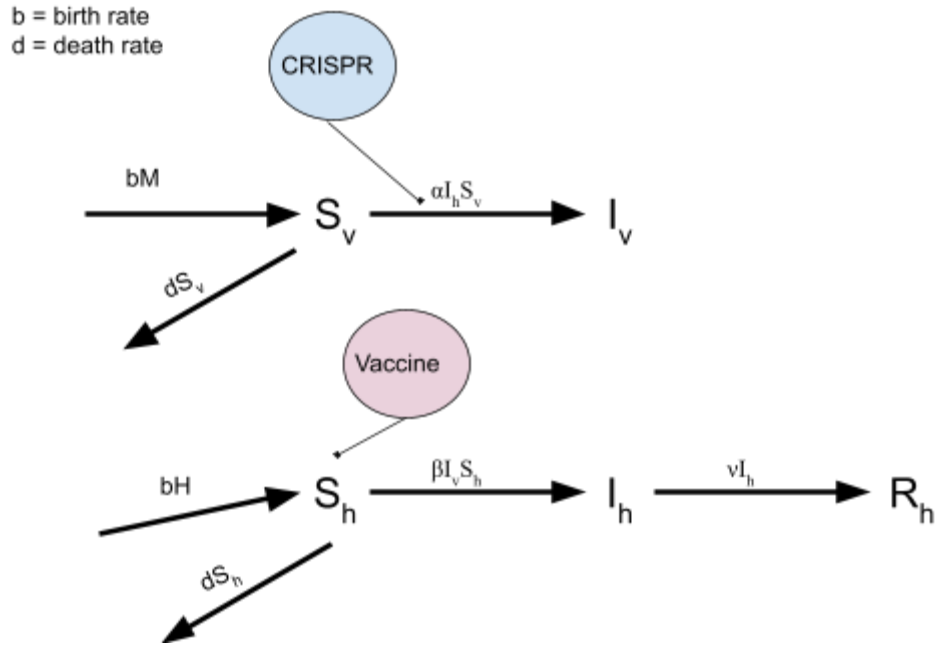

Provide a justification for the connection you made between your interventions and the disease triangle.

| **Intervention** | **Justification for connection** |
|---|---|

| | |
|---|---|
| CRISPR mechanism | It is inhibiting the female mosquito's ability to reproduce, which will decrease the mosquito population |
| Malaria Vaccine | It inhibits humans from getting infected from plasmodium |

## Activity 6: Madagascar treatment SIR model

Edit the basic vector-borne SIR model below showing how the elements of your treatment plan will modify it (double-click to edit). Include births and deaths due to natural causes (humans **and mosquitoes**) **as well as deaths due to disease** (humans). Highlight which stocks/flows will be affected by your treatment(s) with a unique color. Include and define new variables in the table below.



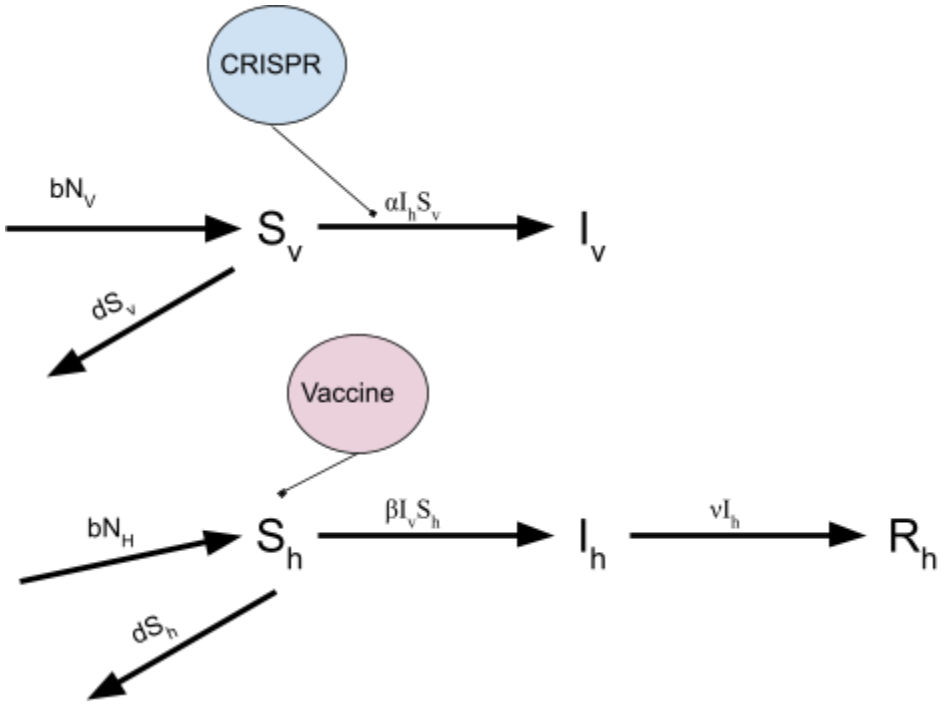

Define any new subpopulations and variables from your stocks and flows diagram in the table below. As a rule of thumb, define a new variable for each intervention that modifies a rate expression in your SIR model.

| **Subpopulation or variable** | **Definition** |
|---|---|
| β | Likelihood of an infected mosquito transmitting to a susceptible human |
| α | Likelihood of an infected human transmitting to an susceptible mosquito |
| v | Frequency of an infected human recovering (1/L) |

| | |
|---|---|
| H | Human |
| V | Vector |
| b | Birth rate |
| d | Death rate |
| N | Population Size |
| | |
| | |
| | |

## Activity 7:  Coding Your Team's Madagascar treatment SIR model

Copy and paste the SIR model from Activity 6 here:




Go to the [Module 7.2  Google Co-laboratory Notebook]:

In Code Block 2, identify the eight rate equations for the various populations in your SIR model. Identify the expressions in the equations that correspond to each of the rate equations in your SIR diagram and paste the relevant expression into the diagram. Do all the expressions in the diagram and the code correspond? Are all the populations accounted for in both the code and your diagram? Are there any variables or subpopulations present in the code that are missing in your table from Activity 6? If so, write them down here:

| **Subpopulation or variable** | **Definition** |
|---|---|
| | |
| | |
| | |
| | |

Run code blocks 1 – 3. What values did you obtain for the following parameters:

<u>Before Interventions</u>

| | |
|---|---|
| `Estimated number infected humans` | |
| `Peak human infections` | |
| `Number of human deaths due to infection` | |
| `Estimated number infected mosquitos` | |
| `Peak mosquito infections` | |

Once you are satisfied that you understand the results in code blocks 2 and 3, modify code block 4 to incorporate the values for the interventions you propose. Introduce any new variables from Activity 6 needed for each intervention that modify the rate equations. Run code block 4 and 5. What values did you obtain for the following parameters:

<u>After Interventions</u>

| | |
|---|---|
| Estimated number infected humans | |
| Peak human infections | |
| Number of human deaths due to infection | |
| Estimated number infected mosquitos | |
| Peak mosquito infections | |

Discuss with your group the results and the significance of the findings. Come up with a conclusion for your intervention modeling.

| |
|---|
| |

## Jigsaw Assignment (Make 6 slides and have one person upload this as a PDF to the assignment link for your team before midnight tonight)

1. **Title Slide** with descriptive title and name of researchers on your diverse expert team
2. **Methods of Intervention**: Include rationales for halting/slowing the malaria epidemic and pros/cons for all interventions
3. **Treatment strategy illustrated in a malaria triangle diagram** (Activity 5): Include effects that caused the malaria outbreak in Madagascar and at least 2 treatment interventions that would reverse future outbreaks. Justify connections between your interventions and the triangle.
4. **SIR diagram with interventions highlighted** (Activity 6): Include new variables as needed and make sure to define any new variables you use. Be sure to include exactly what stock or flow expression is being modified.
5. **Python model** results of the predicted effects of the intervention (Activity 7)
6. **References**: Include all relevant sources used in APA format